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EXAMINER	
KIM, YOUNG J	

ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 06/13/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/634,352	CAO ET AL.	
	Examiner	Art Unit	
	Young J. Kim	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 March 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-46* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-46* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

The present Office Action is responsive to the Amendment received on March 20, 2006.

Preliminary Remark

Claims 1-46 are pending and are prosecution herewith.

Claim Rejections - 35 USC § 103

The rejection of claims 1-13, 23, 24, 27, 29, 32, 33, 36-41, and 43 under 35 U.S.C. 103(a) as being unpatentable over Carulli et al. (Journal of Cellular Biochemistry Supplements, 1998, vol. 30/31, pages 286-296) in view of Russell et al. (U.S. Patent No. 5,861,248, issued January 19, 1999) and Phillips et al. (Methods, 1996, vol. 10, pages 283-288), made in the Office Action mailed on December 19, 2005 is withdrawn in view of the Amendment received on March 20, 2006. Specifically, Applicants are factually correct in stating that none of the cited artisans disclose the step of, "linearly amplifying cDNA and employing the linearly amplified cDNA," in their method.

Rejections, New Grounds

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-13, 23, 24, 27, 29, 32, 33, 36-41, and 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Carulli et al. (Journal of Cellular Biochemistry Supplements, 1998, vol. 30/31, pages 286-296) in view of Russell et al. (U.S. Patent No. 5,861,248, issued January 19, 1999) and Phillips et al. (Methods, 1996, vol. 10, pages 283-288).

Carulli et al. disclose a method of determining differential expression of two or more genes in one or more cells, said method comprising:

a) obtaining population of mRNA from cells and generating a cDNA from said RNA (page 290, 1st column, 1st paragraph), wherein second strand of the synthesized cDNA is labeled with dCTP (Cy3 or Cy5), further wherein the artisan **explicitly state** that linear amplification of the cDNA gives uniform amplification of all transcripts (page 290, 1st column, 2nd paragraph, bottom);

b) contacting the labeled cDNA with an array of probes, specifically, an array having 960 clones of osteoblast EST (page 290, 1st column, 1st paragraph, top);

c) determining the relative hybridization of the at least two genes, which would necessarily involve the determination of relative hybridization of the probes to the linearly amplified population of the labeled aRNA (page 290, 1st column, 1st paragraph, bottom; Figure 2).

With regard to claim 3, Carulli et al. disclose that the cDNA is prepared by reverse transcription (page 290, 1st column, 1st paragraph).

With regard to claims 4, 6-8, and 41, drawn to conditions of incomplete extension reaction, it is determined that the reagents employed by Carulli et al. would eventually be expended, resulting in the halting of the reaction.

With regard to claim 9, the array of probes from osteoblast EST project, thus partly known sequences (page 290, 1st column, 1st paragraph).

With regard to claims 10-13, the detection of hybridization pattern of the samples in the array inherently demonstrates that the probes of the array have hybridized to some region 3' end of the transcript. While the reference is silent in explicitly disclosing that the hybridization occurs at the requisite distance (*i.e.*, claimed) from the 3' end of the transcript, as the PTO does not have the facility to conduct the experiment to determine, whether or not this is, indeed the case, it is

determined that hybridization of the array to a plurality of probes would necessarily result in the hybridization of the probes to their complement transcripts in the requisite distance from their 3' end.

With regard to claim 23, the probes of the array of Carulli et al. would necessarily have a given length.

With regard to claim 24, the array is formed by arraying of a plurality of clones.

With regard to claim 27, the array is disclosed as having the density of greater than 1,000 clones/cm^2 (page 290, 1st column, 1st paragraph).

With regard to claim 29, the clones are arrayed on to glass slides (thus nonporous) (page 290, 1st column, 1st paragraph).

With regard to claims 33, 39, and 40, the labeled cDNA of day-8 osteoblast and the labeled cDNA of day-17 osteoblast and simultaneously hybridized to the array (Figure 2).

With regard to claim 36, the probes bind to differentially expressed genes (Figure 2).

With regard to claim 37, Carulli et al. evidences the identification of the differentially expressed gene (page 290, 1st column, 3rd paragraph), stating, "the clone at E1 was significantly downregulated during differentiation...[t]his gene encodes a novel protein with *sushi* repeats" evidencing that a search had been made.

With regard to claim 38, the cells are from same cell lineage (osteoblast), and the expression profile tranks the different stages of development of the osteoblasts (8 day and 17 day expression profiles).

Carulli et al. do not explicitly disclose that the sample from which RNA is isolated comprises fewer than 1000 cells (claim 1), fewer than 100 cells (claim 5), or more specifically a single cell (claim 2).

Carulli et al., linearly amplifies the cDNA in the form of aRNA (referring to Lockhart et al., page 290, 1st column, 2nd paragraph).

Phillips et al. disclose a method of detecting gene expression from a single cell (Abstract), wherein the method involves the step of obtaining a first population of RNA from a single cell (page 283, 2nd column, 1st paragraph).

Russell et al. disclose a method of generating amplifying cDNAs for the purpose of determining the amount of transcripts, wherein the artisans explicitly disclose:

> "These assay sample the PCR product [in the RT-PCR reaction] in the ***linear portion of the their amplification curves***. The number of PCR cycles that are optimal for sampling must be empirically determined for each target cDNA fragment."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to apply the teachings of Carulli et al. and Russell et al. to monitor the expression of two or more genes from a single cell sample as provided for by Phillips et al., thus arriving at the claimed invention for the following reasons.

The motivation to monitor the gene expression profile at a single cell level is explicitly provided for by Phillips et al.:

"Cell-specific gene expression enables the performance of diverse discrete biological functions in an efficient manner. This specificity is achieved by the coordinated expression of only certain genes in a given cell type. The problem is further complicated when considering ... vast cellular heterogeneity." (page 283, 1st column to 2nd column, 1st paragraph).

Hence, one of ordinary skill in the art at the time the invention was made to apply the teachings of Carulli et al., to examine the gene expression of two or more genes, wherein the

expression pattern is generated from a single cell, for the benefit of achieving the coordinated expression a given cell type without complication produced from cellular heterogeneity.

One of ordinary skill in the art would have had a reasonable expectation of success at combining the teachings of Carulli et al. with the teachings of Phillips et al., as Phillips et al. explicitly disclose a method of isolating RNAs from a single cell, followed by the generation of the corresponding cDNAs (*see* page 284, 2nd column, bottom paragraph for 1st strand cDNA synthesis; *see* page 286, 1st column, 4th paragraph for the second strand cDNA synthesis). The amplification, specifically linear amplification of the isolated RNA from samples has already been contemplated by Carulli et al. as producing better representation of the gene expression as, "linear amplification protocols have been developed to that appear to amplify all transcripts ***uniformly***." (page 290, 1st column, 2nd paragraph), rendering instant claims obvious over the cited references.

With regard to the use of linearly amplified cDNAs in an array hybridization reaction, one of ordinary skill in the art at the time the invention was made would have been motivated to employ any of the known methods of linearly amplifying the gene transcripts, whether it be in the form of an antisense RNA (aRNA; as disclosed by Carulli et al.) or in the form of cDNA (as disclosed by Russell et al.), for the benefit of generating uniformly amplified gene transcripts.

Therefore, the invention as claimed is *prima facie* obvious over the cited references.

Claims 14-22, 25, 26, 28, 30, 31, 34, 35, 42, 44-46 rejected under 35 U.S.C. 103(a) as being unpatentable over Carulli et al. (Journal of Cellular Biochemistry Supplements, 1998, vol. 30/31, pages 286-296) in view of Russell et al. (U.S. Patent No. 5,861,248, issued January 19, 1999) and Phillips et al. (Methods, 1996, vol. 10, pages 283-288) and Phillips et al. (Methods, 1996, vol. 10,

pages 283-288) as applied to claims 1-13, 23, 24, 27, 29, 32, 33, and 36-41 above, and further in view of Lockhart et al[1]. (WO 97/10365, published March 20, 1997).

The teachings of Carulli et al., Russell et al., and Phillips et al. have already been discussed above.

None of the above-recited artisans disclose that their array comprises at least 1,000 genes, or at least 10,000 genes (claims 14 and 15) and their relative expression levels (claims 18 and 19).

None of the above-recited artisans explicitly disclose the use of mismatch probes in their method, though alluding to (page 290, 2nd column, 3rd paragraph, bottom) (claims 16, 17, and 35).

None of the above-recited artisans explicitly disclose a specific fold-difference in expression levels (claims 20-22).

None of the above-recited artisans explicitly discuss fragmentation of the target nucleic acids (claim 25).

None of the above-recited artisans explicitly discuss end labeling (claim 26).

None of the above-recited artisans explicitly discuss the probe length (claim 28).

None of the above-recited artisans explicitly discuss that the samples are from a biopsy or from tissue sample suspected of being neoplastic (claims 30 and 31).

None of the above-recited artisans employ separate hybridization of the first population and second population of cDNA to their respective arrays (claim 34).

None of the above-recited artisans disclose identifying differentially expressed transcripts involving clustering (claim 42).

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[1] Cited previously in Office Action mailed on February 25, 2004.

With regard to claims 14 and 15, Lockhart et al. disclose a method of determining differential expression of a plurality of genes via use of a microarray, said microarray comprising at least 1,000 or at least 10,000 genes (page 4, line 4).

With regard to claims 18-22, the use of microarray of Lockhart et al. would necessarily result in determination of genes that are differentially expressed by the requisite fold.

With regard to claims 16, 17, and 35, Lockhart et al. disclose the use of mismatch probes in order to determine the relative expression level of genes (page 5, lines 24-25), said mismatch of single base occurring at any place within the probe, especially near the center of the probe (page 13, lines 14-22).

With regard to claim 25, Lockhart et al. disclose a fragmentation process of the nucleic acids prior to their application to the array, for the benefit of reducing secondary structures and multiple interactions with close spaced nucleic acid molecules (page 68, lines 5-7).

With regard to claim 26, Lockhart et al. disclose that either the cDNA or cRNA could be labeled during amplification or after amplification process, specifically, end labeling process (page 31, lines 4-5).

With regard to claim 28, Lockhart et al. disclose that the probes of the array range from about 5 to about 45 nucleotides; or 5 to about 50 nucleotides; or 10 to about 40 nucleotides; or 20 or 25 nucleotides in length (page 34, lines 11-16).

With regard to claims 30 and 31, Lockhart et al. disclose that biological sample may be tissues (page 27, line 30), or biopsy samples (page 28, line 2), wherein the method could be employed in detecting differential expression between normal and cancerous cells (page 8, lines 20-21, which would necessarily require the use of a tissues sample being or suspected of being

neoplastic. Absent evidence to the contrary, a fine needle biopsy sample would necessarily have less than 100 cells (meeting claim 5 limitation).

With regard to claim 42, Lockhart et al. disclose a clustering method wherein, the method comprises identification of all genes that have similar expression pattern (or classifying the single cells in clusters determined by similarity of expression profile) (page 21, lines 20-27).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Lockhart et al. with the teachings of Carulli et al., Russell et al., and Phillips et al. to analyze the expression pattern of larger set of genes on a microarray, such as that of Lockhart et al., because by doing so, one of ordinary skill in the art would have been able to capture the advantage of simultaneously monitoring the expression pattern of a plurality of genes (Lockhart et al., page 19, lines 5-15). Additionally, implementing the method of fragmenting the nucleic acid prior to their application onto the microarray, disclosed by Lockhart et al., would have been especially advantageous in applying a large number of samples as the artisans explicitly disclose that fragmentation process provides for the reduction of secondary structure of nucleic acids (to facilitate hybridization process) as well as reducing multiple interactions with close spaced nucleic acid molecules (Lockhart et al., page 68, lines 5-7). While the above-disclosure pertains to the application of labeled cRNA to the microarray, one of ordinary skill in the art would have recognized that the advantage of reducing secondary structure formation and the multiple interactions with close spaced nucleic acid molecules would have been also applicable to cDNAs.

With regard to the use of mismatch probes, Carulli et al. have already disclosed their awareness of the usefulness of mismatch probes, that is, in reducing false hybridization signals, alluding to a disclosure by Lockhart et al. While the artisans have not explicit in whether the technique of employing mismatch probes were implemented, one of ordinary skill in the art would

have been motivated to combine the instant teachings of Lockhart et al. for the advantage of reducing false positive signals by employing mismatch probes.

With regard to claims 44-46, the source of samples from which to isolate the RNA population is obvious in view of the purview of an artisan based on what conditions of what organ the one of ordinary skill in the art would experiment – *i.e.,* cancer of the brain, or other organs, etc.

Therefore, the invention as claimed is *prima facie* obvious over the cited references.

Conclusion

Applicant's arguments with respect to the previous rejections of record have been considered but are moot in view of the new ground(s) of rejection.

No claims are allowed.

Inquiries

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Young J. Kim whose telephone number is (571) 272-0785. The Examiner is on flex-time schedule and can best be reached from 8:30 a.m. to 4:30 p.m. The Examiner can also be reached via e-mail to Young.Kim@uspto.gov. However, the office cannot guarantee security through the e-mail system nor should official papers be transmitted through this route.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Dr. Gary Benzion, can be reached at (571) 272-0782.

Papers related to this application may be submitted to Art Unit 1637 by facsimile transmission. The faxing of such papers must conform with the notice published in the Official Gazette, 1156 OG 61 (November 16, 1993) and 1157 OG 94 (December 28, 1993) (see 37 CFR 1.6(d)). NOTE: If applicant does submit a paper by FAX, the original copy should be retained by applicant or applicant's representative. NO DUPLICATE COPIES SHOULD BE SUBMITTED,

so as to avoid the processing of duplicate papers in the Office. All official documents must be sent to the Official Tech Center Fax number: (571) 273-8300. For Unofficial documents, faxes can be sent directly to the Examiner at (571) 273-0785. Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (571) 272-1600.

Young J. Kim
Primary Examiner YOUNG J. KIM
Art Unit 1637 PATENT EXAMINER
6/12/2006

yjk